41. The method according to claim 1, wherein said labeled probe library from said RNA sample is from the same cell type or tissue from a different individual of the same organism.

42. The method according to claim 32, further comprising said RNA sample contains RNA from different individuals within the same species of organism.

43. The method according to claim 15, further comprising repeating steps (d)-(f) with every sub-group of said one or more groups of members of said non-normalized cDNA library represented in higher amounts by said RNA sample before step (g);

A8 cont.

44. The method according to claim 31, wherein every member of said group members of said non-normalized cDNA library represented in lower amounts by said RNA sample and every member of every sub-group are at least partially sequenced.

45. The method of claim 44, wherein every unique member is pooled.

46. The method according to claim 32, further comprising said RNA sample contains RNA from different developmental stages of the same type of tissue.

REMARKS

Claims 1-8, 10-32 and 37-46 are pending. The specification has been amended to correct certain typographical errors. The claims have been amended to clarify the claim language and to claim certain other aspects of the present invention. The non-elected claims 33-36 were canceled without prejudice to the filing of one or more divisional applications.

The specification was objected to because of a certain typographical error, which has been fixed by the amendment above.

All of the claims were rejected under 35 USC 112, second paragraph, as being indefinite by various recitations. While the examiner noted several allegedly unclear terms and passages, it is clear that the examiner understood the invention. Many claims were amended clarifying the language and others were reworded simply to make them read better. The attached marked-up claims should be of particular help in following the amendments. Specifically, "low" and "high" were changes to the relative terms "lower" and "higher" when describing relative quantities between groups. Also, the different groups were better identified, particularly in claim 15. The nature of the process is somewhat complicated requiring correspondingly complicated and lengthy descriptions; however, these amendments now render all of the claims definite.

Claims 1-5, 7, 10, 11, 13, 14 and 32 were rejected under 35 USC 102(b) as being anticipated by Nelson et al. Nelson et al's method for forming a cDNA library differs from the present invention by certain key differences, which produce a different end result cDNA library.

Nelson et al state that their cDNA library is actually made up of ESTs and other truncated clones. The results in Nelson et al, page 211, second column, lines 6-12 state that many of the clones were less than 300 bp and that this was comparable to the results from other previous cDNA libraries. The remainder of the paper is merely a statement of the results from the ESTs found. See Table I of Nelson et al. Indeed, Nelson et al note that truncation of the cDNA clones is a typical result and are favored from normalization techniques as stated on page 210, first column lines 14-16. See also page 2 of the present specification.

This taught library building is in contrast to the present invention for producing predominantly full-length normalized libraries. Claim 1 recites that the library contains full-length cDNA of genes, not merely short ESTs. Therefore, the present invention is neither taught nor suggested by Nelson et al.

The library in Nelson et al is a library of one tissue, that of a normal human prostate which was extracted from exactly one individual. See page 210, first column,

last paragraph and Nelson et al 1998 (of record). The claimed library is one of many tissues (preferably substantially all) and from different individuals. The goal of the present invention is a complete normalized message library, not just a tissue-specific one. Low abundance cDNA in Nelson et al are low abundance in prostate tissue, which may be high abundance in other tissues. Nelson et al states that they want to use only one tissue to obtain the entire complement of genes expressed by that tissue (line 1) and to avoid the "masking" effects by genes preferentially expressed by other tissues. The present invention uses a different method to make a different set from cDNA, which are low abundance across many tissues. Claims 32 as well as many of the newly added claims 37+ recite the concept of preparing a general message library from multiple tissues, multiple development stages and/or multiple individuals, not a specific tissue library. Note the specification on page 10, lines 8-12 and page 24, first paragraph. When attempting to prepare such a library, the cDNAs that will be considered low abundance will differ and due to the increased variety of mRNA sources. Therefore, not only do the process steps differ but also the desirability of the goal is not suggested by Nelson et al.

Nelson et al is concerned with the tissue defined low-abundance cDNAs as stated in their first sentence and many other places. Therefore, all of the present claims involving multiple sources or use or manipulate the higher abundance cDNAs is clearly outside of and contrary to the teachings of Nelson et al, and thus patentable over the teachings of Nelson et al.

Additionally, claim 1 recites a pooling step as step (f). Firstly, Nelson et al does not pool full-length cDNA clones as they have ESTs. Secondly, Nelson et al selects the clones they want in section 2.2 followed by PCT amplification of the inserts and PCR products were collected. The primers for PCR came from the flanking regions on the vector. Since Nelson et al is only concerned with ESTs, relative short PCR amplicons are acceptable for them. This is a library of amplifiable ESTs, not a library of full-length genes.

PCR has its limitations as to the total length of the region to be amplified. While inserts of only a few hundred nucleotides long could theoretically be amplified, the full-length message of most long inserts would not be amplified by PCR. At a minimum, the

shorter or truncated inserts would preferentially be amplified. This bias would result in a non-normalized library, the opposite from the claimed method result. This is not what the claims recite and present invention is attempting to accomplish by reciting pooling predominantly full-length normalized cDNA library of genes. This is emphasized throughout the specification, for example on page 28, lines 6-10 and page 29, lines 9-14 where applicants report the results of their designed method to bias toward obtaining full-length clones preferentially.

It should also be noted that with differing lengths inserts between Nelson et al and the present invention, the hybridization specificity and efficiency to the labeled probes would differ, also biasing the selection process.

Accordingly, this rejection should be withdrawn, particularly as it pertains to the presently amended claims.

Claim 6 was rejected under 35 USC 103 as being unpatentable over Nelson et al in further view of Somerville et al. To compensate for Nelson et al's lack of preparing a library from plant tissue, the examiner includes Somerville et al to show that one is interested in the plant genome and to assert that it would have been obvious to apply the Nelson et al method for making a plant cDNA library.

However, this combination of references do not compensate for the defects in the Nelson et al reference as described above. Somerville et al is silent and not even concerned with the issues of how to prepare such a library. The 'resulting libraries in Somerville et al are also EST libraries and other partial cDNA sequences. See page 380 column 3, lines 20 – page 381, column 1, line 4. The full-length or near full-length cDNAs mentioned are in the caption of Fig. 2 where only 64 randomly selected clones were compared. No mention of their preparation is provided. As such, Somerville et al adds nothing regarding the independent claims. Accordingly, this rejection should be withdrawn.

Claim 8 was rejected under 35 USC 103 as being unpatentable over Nelson et al in view of El-Meanawy et al. In addition to the teachings of Nelson et al, the rejection

cites El-Meanawy et al to show use of SAGE to construct kidney expression libraries. The examiner contends that it would have been obvious to apply the Nelson et al technique to kidney cells for reasons given in El-Meanawy et al.

The SAGE analysis of a kidney sample in El-Meanawy et al results in a tag expression library. These tags are taught to be only 9-13 bp long. This is clearly not a full-length cDNA clone. Furthermore, only one tissue is analyzed for each library. El-Meanawy et al does not provide for any selection of low abundance RNA or way of reducing the redundancy of high abundance mRNA. Since the goals and purposes in Nelson et al and El-Meanawy et al are opposed, no possible combination of these two references can result, much less anything suggesting the presently claimed invention. Accordingly, this rejection should be withdrawn.

Claims 9 and 12 were rejected under 35 USC 103 as being unpatentable over Nelson et al in view of Frohman et al. Nelson et al has been discussed above. Frohman et al teach RACE as a way for cloning full-length cDNA clones. However, RACE involves a primer which hybridizes somewhere in the middle of the cDNA. To make such a primer, one must know at least part of the mRNA or cDNA sequence. In Nelson et al, nothing is known about the sequences at all which would help in choosing a primer sequence. Therefore, one would not find it obvious to make such a primer. Without a primer, Frohman et al does not provide any teaching as to how to make full-length cDNA clones. Therefore, the Frohman et al RACE method cannot be used in the Nelson et al technique at any point and thus the combination does not render the present claims obvious.

Applicants thank the Examiner for indicating Claims 15-31 to free of the prior art and patentable except for issues of indefiniteness.

CONCLUSIONS

In view of the amendments and comments above, the rejections have been overcome. Reconsideration, withdrawal of the rejections and early indication of allowance are respectfully requested.

The commissioner hereby is authorized to charge payment of any fees under 37 CFR § 1.17, which may become due in connection with the instant application or credit any overpayment to Deposit Account No. 500933.

Respectfully submitted,

Date April 30, 2003

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I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an enveloped addressed to:
Assistant Commissioner for Patents
Washington, DC 20231

On April 30, 2003

Typed or printed name of person signing this certificate John E. Tarcza

Signature

Marked-up claims for

U.S. Serial Number 09/864,637

1. (amended) A method for constructing a normalized full-length cDNA library of genes of low expression, comprising:

(a) constructing a non-normalized cDNA library from an RNA sample, wherein said RNA sample contains different species of RNA of different amounts, wherein said non-normalized cDNA library contains a plurality of members;

(b) separating the members of said non-normalized cDNA library;

(c) constructing a labeled probe library from said RNA sample;

(d) hybridizing a labeled probe library to said non-normalized cDNA library, whereby each individual member of said non-normalized cDNA library is hybridized to [there is] a differential of the amount of labeled probe of said labeled probe library [hybridized to each individual member of said non-normalized cDNA library];

(e) identifying the individual members of said non-normalized cDNA library hybridized with [low] lower amounts of labeled probe; and

(f) pooling the individual members of said non-normalized cDNA library identified in step (e) in a collection;

whereby said collection is said normalized full-length cDNA library of genes of low expression.

2. The method according to Claim 1, wherein said RNA sample is obtained from a cell.

3. The method according to Claim 2, wherein said RNA sample is a mRNA sample.

4. The method according to Claim 2, wherein said cell is an eubacteria, archaebacteria, or eukaryotic cell.

5. The method according to Claim 4, wherein said eukaryotic cell is a plant cell or animal cell.

6. The method according to Claim 5, wherein said plant cell is a soy, tobacco, wheat, rice, or corn cell.

7. The method according to Claim 5, wherein said animal cell is a human, ape, mouse, rat, cow, pig, horse, goat, sheep, dog, cat, chicken, zebrafish, or fruitfly cell.

8. The method according to Claim 7, wherein said human cell is a human kidney cell.

10. (amended) The method according to Claim 1, wherein said constructing of cDNA from said RNA sample comprises catalyzing a reverse transcription reaction for each species of said RNA sample, wherein said catalyzing takes place under conditions permissible for catalyzing a reverse transcription reaction.

11. The method according to Claim 10, wherein said catalyzing comprises:

(i) hybridizing poly-T oligonucleotide primers to said RNA sample;

(ii) adding dATP, dCTP, dGTP, dTTP, and reverse transcriptase; and

(iii) incubating said RNA sample at a temperature permissible for catalyzing a reverse transcription reaction.

12. The method according to Claim 1, wherein said non-normalized cDNA library is a non-normalized full-length cDNA library.

13. The method according to Claim 1, further comprising:

transforming each member of said non-normalized cDNA library into a host cell, wherein said transforming step is subsequent to said constructing and prior to said hybridizing.

14. (amended) The method according to Claim 13, further comprising:

amplifying each member of said non-normalized cDNA library,

wherein said amplifying comprises growing each said host cell containing cDNA, wherein said amplifying step is subsequent to said transforming and prior to said hybridizing.

15. (amended) A method for constructing a normalized cDNA library, comprising:

(a) constructing a non-normalized cDNA library from an RNA sample, wherein said RNA sample contains different species of RNA of different amounts, wherein each member of said non-normalized cDNA library is separate from other members;

(b) identifying the relative amounts of each member of said non-normalized cDNA library represented in said RNA sample;

(c) dividing the members of said non-normalized cDNA library into groups; wherein one group of members of said non-normalized cDNA library is represented in [low] lower amounts by said RNA sample and one or more groups of members of said non-normalized cDNA library is represented in [high] higher amounts by said RNA sample;

(d) selecting [one] a first sub-group of said one or more groups of members of said non-normalized cDNA library represented in [high] higher amounts by said RNA sample;

(e) identifying the members in said group of members of said non-normalized cDNA library represented in higher amounts by said RNA sample, which [that] is not represented within [a] said first sub-group of members selected from said group of members;

(f) forming a second sub-group of members from the members identified in step (e) and repeating step (e) until every member of said group of members of said non-normalized cDNA library represented in higher amounts by said RNA sample has been selected within a sub-group of members;

[(g) repeating steps (d)-(f) with every group of said one or more groups of members of said non-normalized cDNA library represented in high amounts by said RNA sample;

(h)][(g)] pooling the members of said group of members of said non-normalized cDNA library represented in [low] lower amounts by said RNA sample and the members of every sub-group selected in a collection;
whereby said collection is said normalized cDNA library.

16. The method according to Claim 15, wherein said RNA sample is obtained from a cell.

17. The method according to Claim 16, wherein said RNA sample is a mRNA sample.

18. The method according to Claim 16, wherein said cell is an eubacteria, archaebacteria, or eukaryotic cell.

19. The method according to Claim 18, wherein said eukaryotic cell is a plant cell or animal cell.

20. The method according to Claim 19, wherein said plant cell is a soy, tobacco, wheat, rice, or corn cell.

21. The method according to Claim 19, wherein said animal cell is a human, ape, mouse, rat, cow, pig, horse, goat, sheep, dog, cat, chicken, zebrafish, or fruitfly cell.

22. The method according to Claim 21, wherein said human cell is a human kidney cell.

23. The method according to Claim 15, wherein said normalized cDNA library is a normalized full-length cDNA library.

24. (amended) The method according to Claim 15, wherein said constructing a non-normalized cDNA library from an RNA sample comprises catalyzing a reverse transcription reaction for each species of said RNA sample, wherein said

catalyzing takes place under conditions permissible for catalyzing a reverse transcription reaction.

25. The method according to Claim 24, wherein said catalyzing comprises:

(i) hybridizing poly-T oligonucleotide primers to said RNA sample;

(ii) adding dATP, dCTP, dGTP, dTTP, and reverse transcriptase; and

(iii) incubating said RNA sample at a temperature permissible for catalyzing a reverse transcription reaction.

26. The method according to Claim 15, wherein said non-normalized cDNA library is a non-normalized full-length cDNA library.

27. The method according to Claim 15, further comprising:

transforming each member of said non-normalized cDNA library into a host cell, wherein said transforming step is subsequent to said constructing and prior to said identifying of step (b).

28. (amended) The method according to Claim 27, further comprising:

amplifying each member of said non-normalized cDNA library,

wherein said amplifying comprises growing each said host cell containing cDNA, wherein said amplifying step is subsequent to said transforming and prior to said identifying of step (b).

29. The method according to Claim 15, wherein said identifying of step (b) comprises:

(i) constructing a labeled probe library from said RNA sample;

(ii) hybridizing said labeled probe library to said non-normalized cDNA library;

(iii) identifying the relative amounts of labeled probe hybridized to each member of said non-normalized cDNA library.

30. The method according to Claim 15, wherein said identifying of step (e) comprises:

(i) constructing a labeled probe library from said sub-group of members;

(ii) hybridizing said labeled probe library to said group of members;

(iii) identifying each member of said group of members that is not hybridized to by said labeled probe library.

31. (amended) The method according to Claim 15, further comprising:

sequencing [every] a member of said group members of said non-normalized cDNA library represented in [low] lower amounts by said RNA sample and [every] a member of every sub-group selected prior to said pooling, wherein a sufficient number of nucleotides are sequenced to identify members that are represented [by] no more than once; and

pooling [every unique member] unique members determined by said sequencing.

32. (amended) A method for constructing a normalized cDNA library of genes of low –expression in a species, comprising:

(a) constructing a non-normalized cDNA library from an RNA sample from a plurality of different tissues, developmental stages or individuals from the same species of organism, wherein said RNA sample contains different species of RNA of different amounts, wherein each member of said non-normalized cDNA library is separate from other members;

(b) identifying the relative amounts of each member of said non-normalized cDNA library represented in said RNA sample;

(c) pooling the members of [said group of members of] said non-normalized cDNA library represented in [low] lower amounts by said RNA sample in a collection;

whereby said collection is said normalized cDNA library of genes of low expression for a species of organism.

37. The method according to claim 32, wherein RNA from substantially every cell type and/or tissue from the same species of organism is used.

38. The method according to claim 2, wherein said RNA sample is from a plurality of different cell types and/or tissue.

39. The method according to claim 2, wherein said RNA sample is from a plurality of different individuals from the same species of organism.

40. The method according to claim 1, wherein said labeled probe library from said RNA sample is from another cell type or tissue of the same organism.

41. The method according to claim 1, wherein said labeled probe library from said RNA sample is from the same cell type or tissue from a different individual of the same organism.

42. The method according to claim 32, further comprising said RNA sample contains RNA from different individuals within the same species of organism.

43. The method according to claim 15, further comprising repeating steps (d)-(f) with every sub-group of said one or more groups of members of said non-normalized cDNA library represented in higher amounts by said RNA sample before step (g);

44. The method according to claim 31, wherein every member of said group members of said non-normalized cDNA library represented in lower amounts by said RNA sample and every member of every sub-group are at least partially sequenced.

45. The method of claim 44, wherein every unique member is pooled.

46. The method according to claim 32, further comprising said RNA sample contains RNA from different developmental stages of the same type of tissue.

Marked up copy of the second full paragraph on page 3 of the specification.

Sasaki, *et al.* (Sasaki, Y. F., Iwasaki, T., Kobayashi, H., Tsuji, S., Ayusawa, D., and Oishi, M., "Construction of an equalized cDNA library from human brain by semi-solid self-hybridization system", *DNA Res.* 1:91-6, 1996) and Tanaka, *et al.* (Tanaka, T., Ogiwara, A., Uchiyama, I., Takagi, T., Yazaki, Y., and Nakamura, Y., "Construction of a normalized directionally cloned cDNA library from adult heart and analysis of 3040 clones by partial sequencing", *Genomics* 35:231-5, 1996) disclose a method of [eualizing] equalizing an cDNA library by self-hybridizing cDNA with poly(A)$^+$ RNA (with the cDNA in a large excess) and removing the RNA-DNA complexes. This method relies on the RNA-DNA hybridization taking place with all the species and members of the cDNA unseparated.